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DECAPSULATION OF *ARTEMIA* CYSTS: A SIMPLE TECHNIQUE FOR THE IMPROVEMENT OF THE USE OF BRINE SHRIMP IN AQUACULTURE

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ABSTRACT

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Although it is a common practice in different disciplines of fundamental research on the brine shrimp, and despite the very interesting applications that it offers for the use of *Artemia* in aquaculture, the "decapsulation" technique, which removes the outer layer of the cyst shell of *Artemia*, is not known to shrimp and fish aquaculturists.

The present paper describes the technology developed by the authors for the routine decapsulation of *Artemia* cysts. The advantages which result from the use of decapsulated cysts in aquacultural hatcheries are discussed.

INTRODUCTION

While the live nauplii of the brine shrimp *Artemia salina* are excellent food for most fish and crustacean larvae, the non-hatched cysts and their empty shells, if not separated from the nauplii, often cause problems when *Artemia* is used in aquacultural hatcheries. Indeed, the cysts or cyst shells which are ingested by a predator cannot be digested and may cause blockage of the gut or have other deleterious effects (Herald and Rackowicz, 1951; Morris, 1956; Rosenthal, 1969; J.E. Shelbourne, cited by Provasoli, 1969; Stults, 1974). Moreover, as the external surfaces of cyst shells carry spores of bacteria, plant and even animal species (Gilmour et al., 1975; A.S. Agostino, personal communication, 1977), serious infections can occur in fish or crustacean cultures after the addition of mixed suspensions of nauplii and cysts (or cyst shells) (Shelbourne, 1964; MacFarlane, 1969).

For these reasons, when *Artemia* nauplii are used as a live food source in aquaculture, the nauplii are usually separated from the hatching debris. However, the separation techniques are in many cases not very efficient or require the use of special separator boxes (see review by Sorgeloos and Persoone, 1975).

In view of these problems, it is surprising that the technique of Nakanishi et al. (1962), improved by Morris and Afzelius (1967), which removes the outer part of the shell of *Artemia* cysts without affecting the viability of the embryos, has not yet been applied on a large scale for aquaculture purposes.

This study concerns a practical application of *Artemia* cyst decapsulation in aquaculture. Part of the research was carried out at the Tigbauan Station of the Southeast Asian Fisheries Development Center in the Philippines, and the technique described below is now utilized there on a routine basis.

TECHNICAL PROCEDURE

The hard, dark brown, external layer of a cyst, the chorion (Fig.1), which

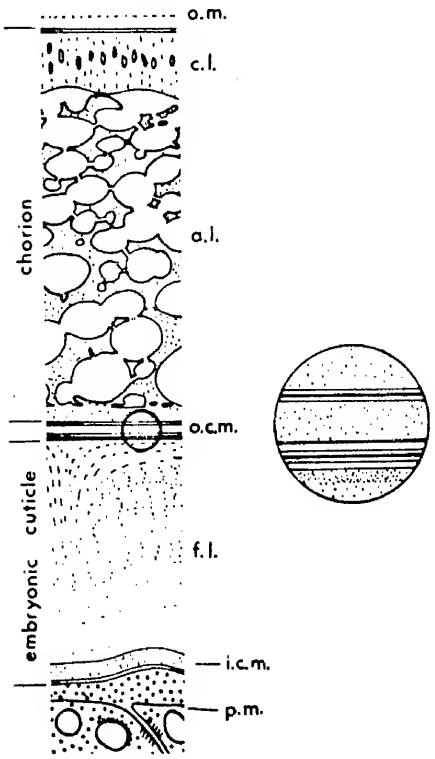

Fig. 1. Composite diagram of shell and membranes in cryptobiotic cysts. o.m., outer membrane; c.l., corical layer; a.l., alveolar layer; o.c.m., outer cuticular membrane; f.l. fibrous layer; i.c.m., inner cuticular membrane; p.m. plasma membrane. (From Morris and Afzelius, 1967.)

can be removed in a hypochlorite solution, is lipoproteinaceous and is impregnated with haematine, a derivate of haemoglobin (Dutrieu, 1960; Linder, 1960; Anderson et al., 1970). It has numerous interconnected canals which are filled with air and are in contact with the surface of the cortical layer. According to Mathias (1937) this alveolar layer contributes to the buoyancy of the cyst.

The dry cysts are hydrated in a funnel-shaped container (minimum ratio of height to width of the water column is 7 : 3) with tap water or sea water and kept in continuous suspension by aeration from the bottom. After 1 h, the suspension is diluted with an equal volume of commercial hypochlorite ("Chlorox", "Eau de Javel", "Oldrox", "Sanichlor" or another brand) to obtain a final concentration of active ingredients of 2.12% (most commercial brands contain 5.25% active ingredients). The oxidation process starts immediately and, as the chorions dissolve, a gradual colour change is observed in the cysts from dark brown via white to orange.

Within 7—10 min, the chorions disappear completely and the decapsulated cysts should then be filtered immediately and thoroughly washed with tap water or sea water in order to remove all traces of hypochlorite. The treated cysts are now either incubated directly for hatching or, after immediate dehydration in a brine solution, stored for later use. The dehydration is performed by transferring the decapsulated cysts into a saturated solution of sodium chloride in tap water or sea water. After agitation by bubbling air through the solution for 3—4 h at room temperature, the dehydrated cysts are concentrated and distributed into smaller vials, containing a saturated brine solution. Studies undertaken to determine the most appropriate conditions for storage of decapsulated cysts revealed that the hatching efficiency of cysts (exposed to optimum hatching conditions) does not decrease with storage if decapsulated cysts are stored at temperatures of $-4°C$ or lower (maximum preservation period tested to date: 8 weeks).

While studying the possibility of decapsulating cysts at high densities, substantial temperature increases were experienced in the medium during the oxidation process and this limits the density and the total quantity of cysts that can be treated in one single container without affecting the viability of the embryos. From previous research, however, we know that as long as the temperature of the medium is kept below 40° C, the hatching efficiency remains maximal (Sorgeloos et al., 1976).

The standard technique that has been worked out assures successful treatment at any density not exceeding 1 g/15 ml tap water or sea water and with no more than 200 g of cysts. It should be mentioned that this procedure was developed in a tropical climate where temperatures of the tap water are up to 27° C.

Decapsulation of larger quantities of cysts is possible either at lower cyst densities or with cooling of the suspension of hydrated cysts to keep the temperature of the medium during the decapsulation process below 40° C; for example, 1 kg of San Francisco Bay cysts can be successfully decapsulated in

30 l medium if the water is cooled with ice to 15° C prior to addition of the hypochlorite (15 l).

Although we have not tested this out for *Artemia* yet, Belk (1970) has demonstrated that conchostracan cysts from which the thick outer shell has been removed are very sensitive to the ultraviolet light of the sun. Until this has been tested for treated cysts of *Artemia*, they should be protected from irradiation by the sun during the decapsulation process and incubation for hatching.

DISCUSSION

The advantages of decapsulation for the practical use of brine shrimp in aquaculture are quite obvious:

(1) Separation of the nauplii from the hatching debris is superfluous since the only remainder after hatching of decapsulated cysts are thin, transparent membranes: the embryonic cuticles.

(2) Disinfection of the cysts through the hypochlorite treatment.

(3) Possible direct ingestion and digestion of decapsulated cysts by fish and crustacean larvae.

Next to the enormous advantage of making the tedious and cumbersome separation of nauplii and cyst shells after hatching superfluous, with the inherent avoidance of all types of contamination, it also appears that some crustacean larvae (*Penaeus monodon*), fish larvae (*Poecilia reticulata; Solea solea*; C. Claus, personal communication, 1977) and a few other marine species (K. Katsutani, personal communication, 1976) can be fed directly with decapsulated cysts. It should be emphasized, however, that after the decapsulation treatment a cyst sediments out of suspension as a result of the loss of the chorion which, as mentioned previously, ensures the buoyancy of the cyst.

Experiments are now in progress to determine more precisely the rates of uptake of these inert (non-hatched) decapsulated cysts by different predators and their digestibility in comparison to live nauplii.

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